THE INFLUENCE OF URINE PH CHANGES ON THE RENAL EXCRETION OF WEAKLY ACIDIC DRUGS

BY

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SUMMARY

This project was carried out to investigate the influence of urine pH changes on the renal excretion of drugs that are weakly acidic. In choosing the drugs to be used in the project the practicability of their assay in a common laboratory was considered. Hence four drugs were chosen, Aspirin, Chlorothiazide Sulphathiazole and Sulphafurazole. Rats were used in all the experiments that were carried out. In each experiment the urine was collected over a period of 24 hours and analysed for drug in question. In this way, the amount of drug excreted in urine under varying urine pH conditions were determined. Sodium bicarbonate and Ammonium chloride were used to make the urine alkaline and acidic respectively.

INTRODUCTION

This project was designed to determine the effects of oral administration of alkali and acid upon the renal excretion of drugs that are weakly acidic.

The kidney is the most important organ of excretion and as such most substances are excreted in urine. However, some other substances are excreted in bile, sweat, saliva, gastric juice, or from the lungs. The excretory unit of the kidney is the Nephron. In the human kidney there are millions of these units. The nephron essentially consists of a filter called the Bowman's Capsule, with a long stem called a renal tubule. The collecting duct is also functionally a part of the nephron. The blood vessels that supply the capsule oaand the tubule are also an essential part of the nephron. The Bowman's Capsule is packed with a mass of branching inter connecting capillaries (glomerular tuft) which provides a large surface area of capillary endothelium through which fluid and small molecules may filter into the capsule and pass down the tubule. The glomerular tuft together with the Bowman's capsule constitute the glomerulus. The glomerular capillary endothelium and the supporting layer of Bowman's capsule

have pores ranging upwards to 40°A. Hence, unbound solutes (drugs) and even a little amount of albumin pass into the glomerular filtrate.

The post-glomerualr vessels which lie close to the tubules are critically important to renal function in the sense that substances re-absorbed from the filtrate by the tubules are returned to the blood along these vessels. Functionally the tubule can be divided into three major parts, the proximal convoluted tubule, the loop of Henle and the distal Convoluted tubule.

After filtration the glomerular filtrate passes through the proximal tubule, where some solutes may be re-absorbed through the tubular epithelium and returned to the blood stream. Re-absorption occurs partly by passive diffusion and partly by active transport especially with sodium and glucose. Consequently, by these processes the filtrate becomes diminished in volume by approximately 80% in the Proximal tubule, although it is not concentrated. In the Proximal tubule some acidification occurs as a result of carbonic anhydrase activity in the tubule cells and the diffusion of hydronium ion reacts with the bicarbonate ion, which is converted to re-absorbable non-ionic carbon dioxide.

There is also active transport of organic cations and ions into the lumen (tubular secretion), each by a

a separate system. These active transport system are extremely important in the excretion of a number of drugs, for example Penicillin G is rapidly secreted by the anion transport system and tetraethylammonium by the cation transport system.

As the filtrate travels down the tubule through the loop of Henle it becomes concentrated, especially at the bottom, as a result of active reabsorption and the counter current distribution effect of the renal apparatus. In the distal tubule sodium re-absorption occurs partly in exchange for Potassium and hydronium ions. Ammonia secretion may be either acidified or alkalinised according to the acid-base and electrolyte requirement.

Drugs are also re-absorbed in the distal tubule and the pH of the urine here is extremely important in determining the rate of re-absorption and amount re-absorbed. The pH of the tubular fluid also affects the tubular secretion of drugs. When the drug in the tubule is highly ionised only a little of the drug can be reabsorbed. Hence most of the drug is rapidly excreted in urine. The urine pH and hence drug excretion may fluctuate widely according to the diet, exercise, drugs, time of day and other factors.

Obviously, the excretion of weak acids and bases can be partly controlled with acidifying or alkalinizing salts for example Ammonium Chloride or Sodium bicarbonate respectively.

Comparative studies on potency and efficacy of drugs in man have demonstrated the importance of controlling urinary pH. Urine pH is important only when the drug in question is a weak acid or base of which a significant fraction is excreted through the renal route. The plasma levels of the drug will be affected due to the changes in the excretory rate. This may have far reaching effects on the therapeutic effect of the drug.

The importance of urine pH, in the excretion of drugs has been illustrated by several workers. Milne et al (1957) found that mecamylamine (basic) is excreted more than four times faster when the urine pH is less than 5.5 than when it is above 7.5. Haag and Larson (1942) demonstrated that in the case of nicotine the extent of urinary excretion of the chemical may be related to the pH of the urine. They emphasized the importance of taking into consideration the dissociation constant of a drug and the relative reabsorbility of the free and dissociated base. Extending these studies to the urinary excretion of quinine in man, Haag, Larson and Ehwarts (1943) found that the urinary [illegible] could be doubled by passing from an alkaline to an acidic urine and they ascribed the difference to greater

re-absorption of quinine from the urinary tract when the urine is alkaline. Emerson and Dole (1943) found that renal clearance of quinacrine was subject to 100-fold variations due principally to two variables, the urinary pH and the renal plasma flow. The army malaria research unit at Oxford (1945) emphasised the striking parallelism between excretion of Quinacrine and Ammonia.

The effect of Ammonium Chloride and Sodium bicarbonate on the urine pH

After ingestion and absorption, the NH_4^+ ion is converted by the liver to Urea, during which process hydrogen ions are liberated.

$$NH_4^+ \longrightarrow NH_3 + H^+ + Cl^-$$

$$NH_3 \longrightarrow \text{Urea.}$$

The hydrogen ion so formed reacts with bicarbonates and other buffers in the extra-cellular fluid. Reduction in the bicarbonate concentration causes an increase in $\left[\frac{H_2CO_3}{HCO_3^-}\right]$ ration ($H_2CO_3 \rightleftharpoons HCO_3^- + H^+$) thus causing an increase in the concentration of hydrogen ions in the extra-cellular fluid. Consequently there is a fall in the pH, resulting in the formation of acidic urine. The end result is that chloride ion displaces the bicarbonate ion,

the latter is converted to carbon dioxide. Thus, the chloride to the kidneys is increased and appreciable escapes reabsorption along with an equivalent amount of cation (mainly Na^+) and isosmotic quantity of water.

After ingestion of sodium bicarbonate, the excess of carbonate load presented to the distal tubule is not completely neutralized by the available hydrogen ions. Consequently the presence of excess bicarbonates in the extra-cellular fluid result in the production of alkaline urine.

MATERIAL AND APPARATUS

(i) Alkalinising agent - Sodium bicarbonate - (BDH)

(ii) Acidifying agent - Ammonium chloride - Analytical reagent

(iii) Drugs used:-

Aspirin Tablets, 300mg B.P (Boots Pharmaceuticals Ltd)

Sulphafurazole Tablets, 500mg. B.P (Clough England)

Sulphathiazole Tablets, 500mg. B.P.C (Clough England)

Chlorothiazide Tablets, 500mg B.P (E.T. Monk and Co. Ltd)

(iv) Animals - Rats

(v) Flasks and burette

(vi) Metabolic cages.

INSTRUMENTS

Ultraviolet Spectrophotometer - Unicam SP 800

PH meter. - Pye Unicam

EXPERIMENTAL

PROCEDURE:

Determination of amount of acidifying and alkalinizing agents

The purpose of these set of experiments was to determine the approximate amount of either Ammonium chloride or Sodium bicarbonate required to achieve acidic and alkaline urine pH values respectively in rats. In each case increasing amounts of either Ammonium Chloride or Sodium bicarbonate were orally fed to the rats and the urine collected over a period of 24 hours. The pH of the urine obtained was determined using a pH meter. This exercise was repeated until the minimum amounts of either Ammonium chloride or Sodium bicarbonate required to produce expected urine pH was obtained. (In this case the expected urine pH was either acidic or alkaline) The results are shown on Table I.

Quantitative Estimation of Drugs in Urine under Varying Urine pH

Three groups of rats each consisting of 3 animals were chosen and starved for at least 24 hours. Three rats were used for each experiment to ensure enough urine was obtained. The rats were hydrated to ensure reasonable amount of urine was collected within the experimental period. To one of the groups Sodium bicarbonate was given orally and to the second group Ammonium Chloride was given. The

The third group was used as a control. Then equal amounts of the drug were given orally to the three groups of rats. The three groups of rats were then placed in three different metabolic cages and the urine collected in measuring cylinders over a period of 24 hours. The pH of the urine was noted to ensure it was within the expected range. The urine was filtered and decolourised with charcol. The urine was then quantatively analysed for the drug under test. This was done by obtaining an absorption spectra for the drug. 0.1N sulphuric acid and 0.1N sodium hydroxide were used as the solvents to obtain the absorption spectra of Aspirin and Chlorothiazide respectively. Typical absorption spectra are shown on figures 1 and 2. The results obtained are shown on Tables II and III.

Quantative estimation of Sulphafurazole and Sulphathiazole in Urine

The experimental set up was the same as for Aspirin and Chlorothiazide just described except that the method of analysis was titrimetric. A titration was carried out with 0.1M standard Sodium Nitrite solution at a temperature below 15°C until a drop of solution immediately gives a blue colour on starch-Iodide paper. The end point was complete when the end point could be reproduced after the titrated solution was allowed to standard for one minute. The titre was noted. The results obtained are shown on Tables IV and V.

R E S U L T S

Table I: Determination of amount of acidfying and alkalinising agents

	SODIUM BICARBONATE			AMMONIUM CHLORIDE		
Amount given to each rat	70mg/ 0.25Kg.	140mg/ 0.25Kg.	210mg/ 0.25Kg.	300mg/ 0.25Kg.	60mg/ 0.25Kg.	90mg/ 0.25Kg.
Volume of urine collected	19ml.	23ml	21ml	21ml	22ml	20ml

Jailer, J.W, Rosenfeld, J. and Shannon, J.A in their work on renal excretion of quinacrine, Chloroquine and Santaquine used sodium bicarbonate and Ammonium Chloride to make urine alkaline and acidic respectively.

Calculation of drug in urine from absorbance:

The spectrophotometric method was used to analyse the urine for Aspirin and Chlorothiazide. Since the extinction Coefficients for both drugs at their λ max is known, it is possible to calculate their concentration in urine using the Beer-Lambert relationship.

$$A = E_{1\%_{1cm}} \; C \; X \; l$$

Where A = Absorbance at max

$E_{1\%_{1cm}}$ = Extinction Coefficient at max

C = Concentration in g/100 ml

l = Path length of the cell in Centimetres.

For example, the amount of Aspirin excreted in urine under alkaline conditions was calculated as shown below:-

$$A = E_{1\%1cm} \; C \; X \; 1$$

$$0.63 = 65.5 \; X \; C \; X \; 1$$

$$C = \frac{0.63}{65.5} \; g/100ml$$

$$C = 0.00962g$$

$$C = 9.62mg \text{ of aspirin}$$

Similarly, the amount of Chlorothiazide excreted in alkaline urine can be calculated:-

$$A = E^{1\%}_{1\,cm} \times C \times 1$$

$$0.92 = 700 \times C \times 1$$

$$C = \frac{0.92}{700} \text{ g/100ml}$$

$$C = 0.001314\text{g}$$

$$C = 1.314\text{mg of Chlorothiazide.}$$

But since a dilution of ten-fold was made during the analysis then concentration of Chlorothiazide in urine

$$= 13.14\text{mg.}$$

The other values were similarly calculated as shown above. The results are shown in tables II and III.

Table II

ASPIRIN

	PH	Amount of drug given	Absorbance at λmax 276 nm	Amount of drug exoreted in urine	Average amount of drug exoreted in urine
Sodium bicarbonate	8.7	300mg	0.70 0.56	10.69mg 8.55mg	9.62mg.
Ammonium Chloride	5.6	300mg	0.15 0.135	2.29mg. 2.069mg	2.18mg.
Control (no agent)	7.1	300mg	0.29 0.395	4.43mg 6.03mg	5.23mg.

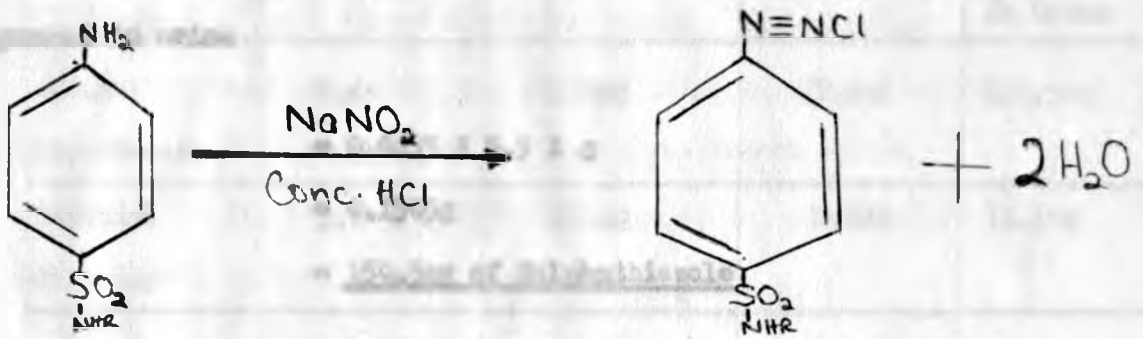
Table III

* CHLOROTHIAZIDE

	PH	Amount of drug given	Absorbance at λ_{max} 228	Amount of drug excreted in urine
Sodium bicarbonate	8.5	40mg	0.92	13.14Mg.
Ammonium Chloride	5.7	40mg	0.80	11.4Mg.
Control (no agent)	7.2	40mg	0.89	12.7Mg.

* A ten-fold dilution was made

In calculating the amount of Sulphathiazole or Sulphafurazole excreted the following relationship was derived. From the titration reaction, 1 mole of Sodium Nitrite is required for every mole of either Sulphathiazole or Sulphafurazole.



$Ar\text{-}NH_2 \xrightarrow[\text{Conc. HCl}]{NaNO_2} Ar - N \equiv NCl + 2H_2O$

Therefore

1 molar solution of $NaNO_2$ = 255g of $C_9H_9N_3O_2S_2$ (Sulphathiazole)

1000ml of 1N $NaNO_2$ = 255g of $C_9H_9N_3O_2S_2$

1 ml of 1N $NaNO_2$ = 0.255g of $C_9H_9N_3O_2S_2$

1 ml of 0.1N $NaNO_2$ = 0.0255g of $C_9H_9N_3O_2S_2$

255g = Molecular weight of Sulphathiazole

Similarly, a similar relationship can be derived for Sulphafurazole:-

1 molar solution of $NaNO_2$ = 267.3g of $C_{11}H_{13}N_3O_3S$

1000ml of 1N $NaNO_2$ = 267.3g of $C_{11}H_{13}N_3O_3S$

1 ml of 1N $NaNO_2$ = 0.2673g of $C_{11}H_{13}N_3O$

1 ml of 0.1N $NaNO_2$ = 0.02673g of $C_{11}H_{13}N_3O_3S$

267.3g = Molecular weight of Sulphafurazole.

The above relationships were used to calculate the amount of drug excreted in urine as follows:-

For example in, alkaline condition, the amount of Sulphathiazole passed in urine

= 0.0255 X 5.9 X g

= 0.150g

= 150.5mg of Sulphathiazole

Similarly, the amount of Sulphafurazole excreted in alkaline condition, can be calculated:

= 0.0263 X 6.8 g

= 181.76mg of Sulphafurazole

The other values were similarly calculated as shown above. The results are shown in tables IV and V.

Table IV

SULPHATHIAZOLE

	PH	Amount of drug given	Titre	Amount of drug excreted in urine
Sodium bicarbonate	8.4	180mg	5.9ml	150.5mg
Ammonium Chloride	5.7	180mg	2.8ml	71.5mg
Control (No agent)	7.0	180mg	4.1ml	104.55mg

Table V

SULPHAFURAZOLE

	PH	Amount of drug given	Titre	Amount of drug excreted in urine
Sodium bicarbonate	8.7	210mg	6.8ml	181.76mg
Ammonium Chloride	5.5	210mg	3.5ml	113.56
Control (no agent)	7.1	210mg	5.4ml	144.34mg

DISCUSSION

ASPIRIN

In the body Aspirin (acetylsalicylic acid) is hydrolysed to Salicylic acid. Hence, due to the presence of this metabolite in urine the estimation of aspirin in urine could not be done using a direct titration method. Consequently a spectrophotometric method was adopted to discriminate between the two. Aspirin and Salicylic acid do not absorb at the same λmax in UV, aspirin absorbs at λmax 276nm, 0.1N Sulphuric acid is used as the solvent.

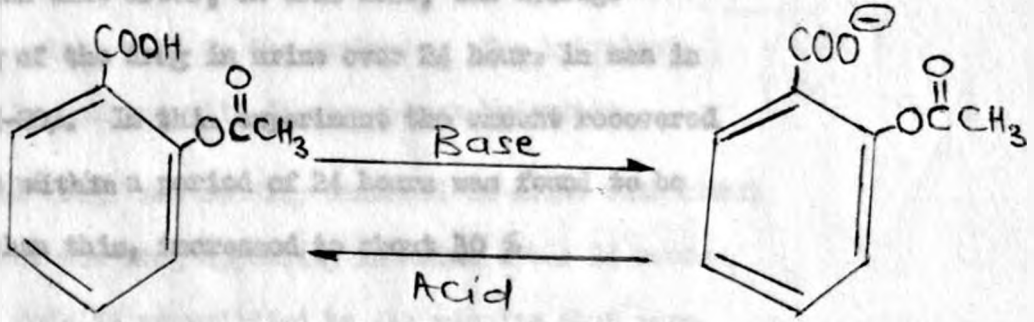
From the results obtained, shown on table II, it is apparent that more Aspirin is excreted in urine under alkaline condition compared to the amount excreted under acidic or normal conditions. This is in keeping with what would be expected - the least amount being excreted under acidic conditions.

This result can be explained by considering the Chemistry of the acetyl Salicylic acid molecule and the mechanisms of excretion in the kidney. Aspirin being an acidic drug will readily ionize under alkaline conditions. In this state, the Aspirin is less lipid soluble and hence not readily absorbed through biological membranes. Therefore, alkalinizing the tubular fluid by ingesting Sodium bicarbonate the aspirin will be ionized to some extent. Consequently, in this form, very little of the aspirin in the tubules will be reabsorbed back into the blood stream. As such more aspirin will appear in urine.

Conversely, under acidic conditions very little of the drug will be in the ionized form, resulting in more drug being reabsorbed back into the blood stream from the renal tubules. Hence there will be a decrease in the amount of drug appearing in urine.

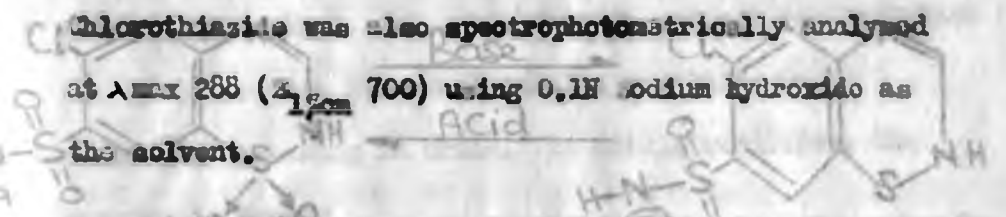
By alkalinising the urine the amount of aspirin excreted in urine increases to about 3 - 4%, whereas the amount excreted in acidic condition is about 1%. Aspirin and other salicylate are rapidly distributed to all body tissues. This may explain why only a small amount is excreted in urine. The rate of excretion of aspirin varies with the pH of the urine, increasing as the pH rises and being greatest at pH 7.5 and above.

Ionization Equilibrium of Aspirin



CHLOROTHIAZIDE

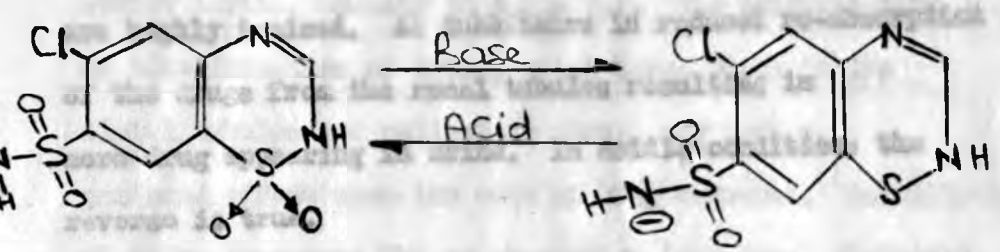
Chlorothiazide was also spectrophotometrically analysed at λ_{max} 288 (A_{1cm} 700) using 0.1N sodium hydroxide as the solvent.



The sulphamido group in chlorothiazide makes the molecule slightly acidic. Consequently, under very alkaline conditions it will ionize to some extent by loosing a proton. This ionization takes place only to a limited extent. Hence very little of the drug will be in the ionised form. This explains why there was only a small variation in the amounts of the drug excreted in urine by varying urine pH.

By alkalinising the urine there is only a small increase in the amount of the drug excreted in urine compared to the amount excreted in acidic conditions. It has been shown that after, an oral dose, the average recovery of the drug in urine over 24 hours in man is about 15-20%. In this experiment the amount recovered in urine within a period of 24 hours was found to be higher than this, increased to about 30 %.

Ionisation equilibrium of chlorothiazide



Sulphafurazole and Sulphathiazole

The quantative estimation of these drugs involves a diazotization reaction which involves the reaction of a primary aromatic amine with Nitrous acid to form a diazonium salt.

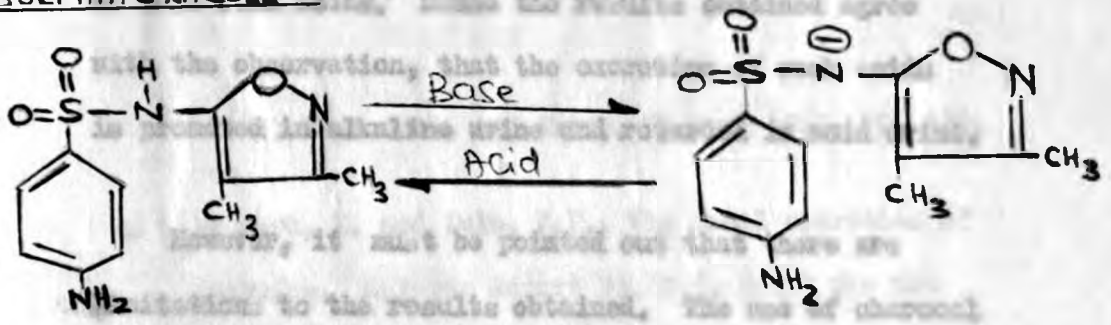
In the body these drugs are readily acetylated, to form conjugated derivatives. Hence in analysing the urine for these drugs, the drugs have to be subjected to an hydrolytic reaction to unmask the primary amine which is made use of in the analysis. The hydrolysis was done by boiling with dilute sodium hydroxide.

These drugs are readily excreted in urine with about 90 - 95% of the drug appearing in urine after 24 hours in man. This is exemplified by the results that were

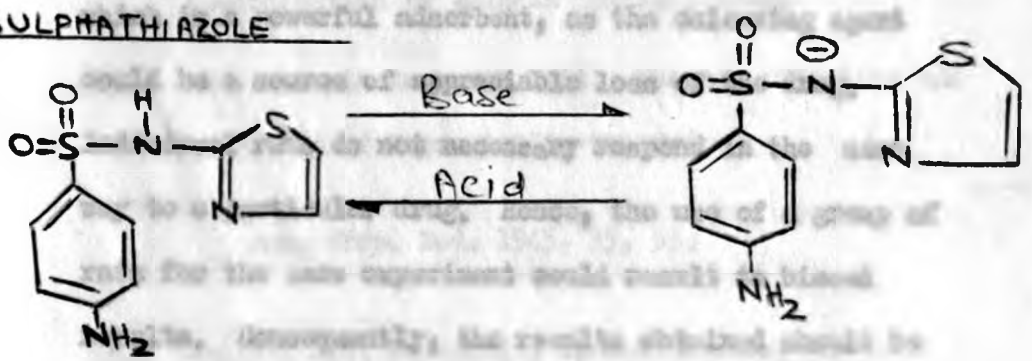
obtained in this experiment. By alkalinising the urine there was an appreciable increase in the amount of the drugs excreted in urine. This can be explained by the fact that under these condition the drugs are highly ionized. As such there is reduced re-absorption of the drugs from the renal tubules resulting in more drug appearing in urine. In acidic condition the reverse is true.

Ionization Equilibrium for sulphathizole and sulphafurazole

SULPHAFURAZOLE



SULPHATHIAZOLE



It must be noted, that explanations advanced for the results obtained only apply for the drug that has been absorbed from the gastro-intestinal tract and has been filtered in the kidney into the renal tubule.

CONCLUSION

The pH of urine is normally maintained within fairly strict limits, usually pH 6.8 - 7.4. The acidification or alkalinization of urine, which takes place in the distal tubules and collecting ducts, may have a profound effect upon the rate of drug excreted. Acidification of urine increases the re-absorption (and thus diminishes the excretion) of weak acids. Conversely, alkalinization decreases the reabsorption (and thus increase excretion) of such weak acids. Hence the results obtained agree with the observation, that the excretion of weak acids is promoted in alkaline urine and retarded in acid urine.

However, it must be pointed out that there are limitations to the results obtained. The use of charcoal which is a powerful adsorbent, as the colouring agent could be a source of appreciable loss of the drug. Individual rats do not necessary respond in the same way to a particular drug. Hence, the use of a group of rats for the same experiment could result to biased results. Consequently, the results obtained should be accessed in the light of these factors.

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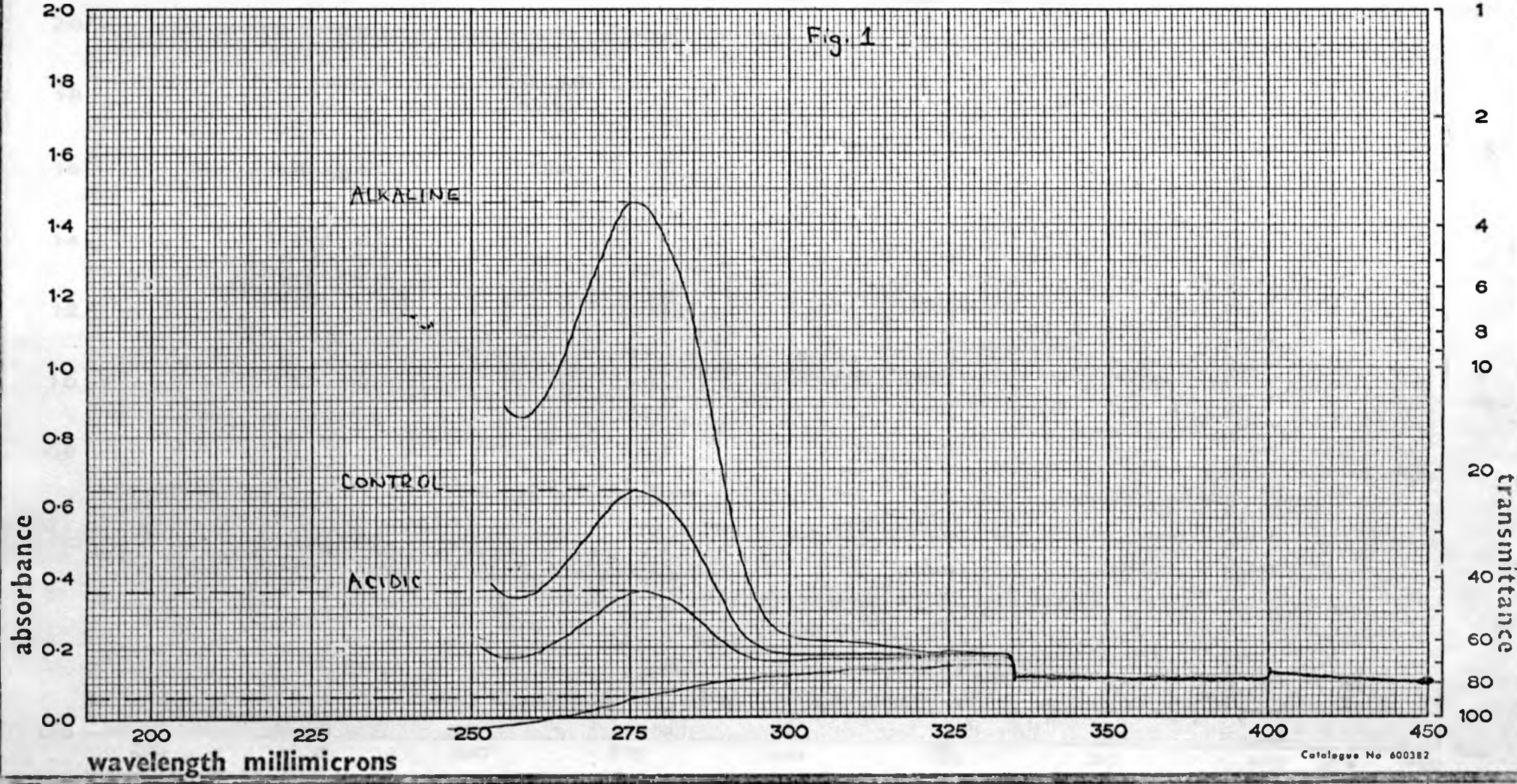
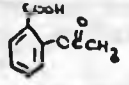
Fig. 1



Catalogue No 600382

ALIGN WITH INDEX ON THE RECORDER

SAMPLE AND FORMULA



CONCENTRATION

REFERENCE 0·1N H_2SO_4

PATH LENGTH 1 cm MM.

SCANSPEED FAST ☑ SLOW ☐

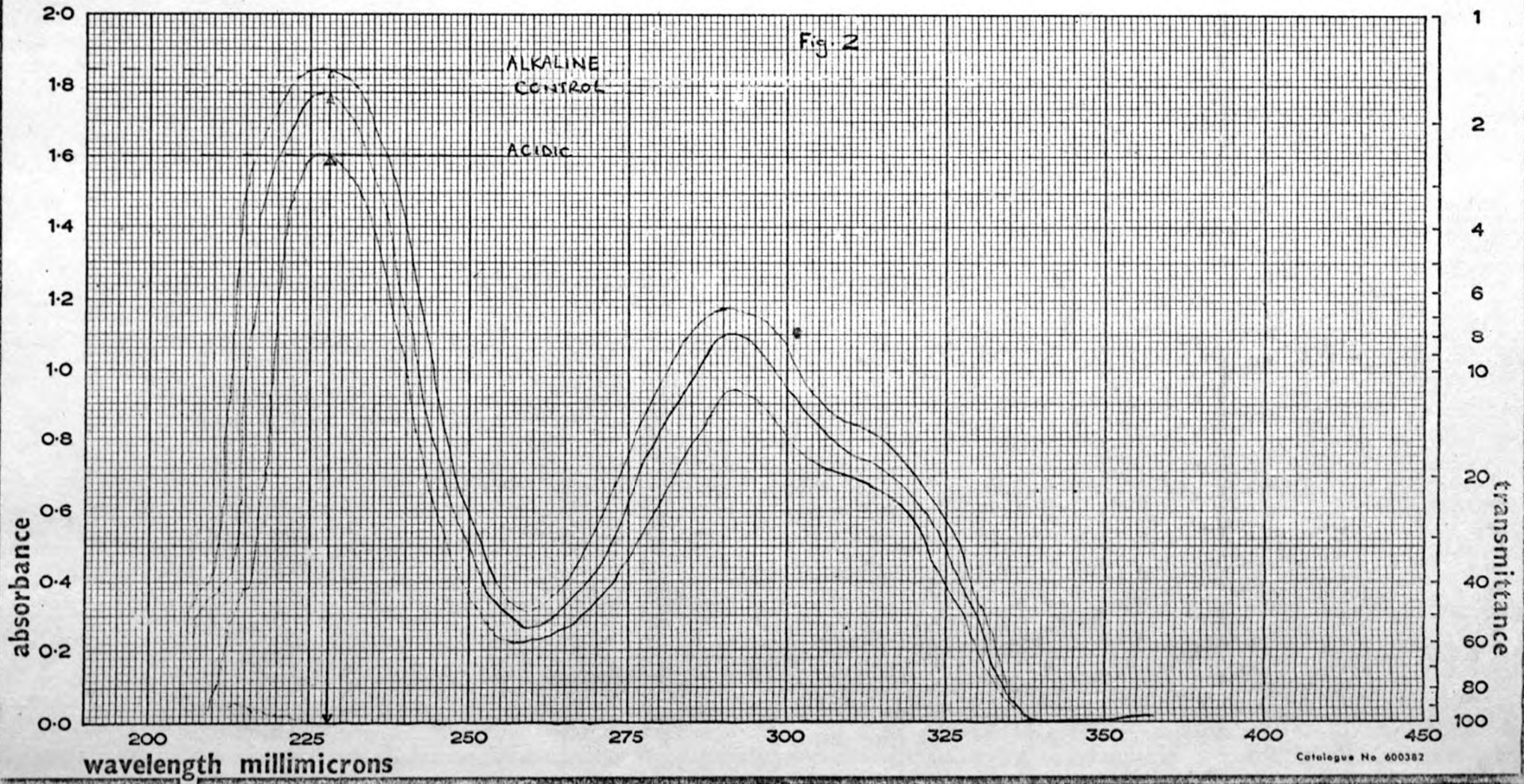
DATE

OPERATOR MUTUNGI, S·K·

REF. NO.

UNICAM SP 800

Fig. 2



Catalogue No 600382

ALIGN WITH INDEX ON THE RECORDER

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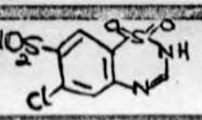

CONCENTRATION

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PATH LENGTH 10 MM.

SCANSPEED FAST ☑ SLOW ☐

DATE 16-2-79

OPERATOR MUTUNGI, S·K

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